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* * * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 16:49:30 ON 23 JUN 2006

=> fil .bec
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.21       0.21

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS,
       ESBIOBASE, BIOTECHNO, WPIDS' ENTERED AT 16:49:43 ON 23 JUN 2006
ALL COPYRIGHTS AND RESTRICTIONS APPLY.  SEE HELP USAGETERMS FOR DETAILS.

11 FILES IN THE FILE LIST

=> s alpha(w)amylase(5a)bacillus(5a)(muta? or variant#)
FILE 'MEDLINE'
        558654 ALPHA
         16202 AMYLASE
         52343 BACILLUS
        519956 MUTA?
        114021 VARIANT#
L1          32 ALPHA(W)AMYLASE(5A)BACILLUS(5A)(MUTA? OR VARIANT#)

FILE 'SCISEARCH'
        790556 ALPHA
         18093 AMYLASE
         53718 BACILLUS
        505178 MUTA?
        126730 VARIANT#
L2          36 ALPHA(W)AMYLASE(5A)BACILLUS(5A)(MUTA? OR VARIANT#)

FILE 'LIFESCI'
        172262 ALPHA
          4593 AMYLASE
         27437 BACILLUS
        229719 MUTA?
         38565 VARIANT#
L3          27 ALPHA(W)AMYLASE(5A)BACILLUS(5A)(MUTA? OR VARIANT#)

FILE 'BIOTECHDS'
         31302 ALPHA
          5942 AMYLASE
         18553 BACILLUS
         46339 MUTA?
         15892 VARIANT#
L4           71 ALPHA(W)AMYLASE(5A)BACILLUS(5A)(MUTA? OR VARIANT#)

FILE 'BIOSIS'
        694816 ALPHA
         28512 AMYLASE
         71451 BACILLUS
        561202 MUTA?
        115575 VARIANT#
L5          48 ALPHA(W)AMYLASE(5A)BACILLUS(5A)(MUTA? OR VARIANT#)

FILE 'EMBASE'
        620236 ALPHA
         16565 AMYLASE
         38236 BACILLUS
        434624 MUTA?
         99436 VARIANT#
L6          28 ALPHA(W)AMYLASE(5A)BACILLUS(5A)(MUTA? OR VARIANT#)
```

```
FILE 'HCAPLUS'
       1615721 ALPHA
         45665 AMYLASE
         90542 BACILLUS
        530340 MUTA?
        113151 VARIANT#
L7         136 ALPHA(W)AMYLASE(5A)BACILLUS(5A)(MUTA? OR VARIANT#)

FILE 'NTIS'
         29097 ALPHA
           158 AMYLASE
          1732 BACILLUS
         10117 MUTA?
          4679 VARIANT#
L8            0 ALPHA(W)AMYLASE(5A)BACILLUS(5A)(MUTA? OR VARIANT#)

FILE 'ESBIOBASE'
        239105 ALPHA
          4844 AMYLASE
         17897 BACILLUS
        267301 MUTA?
         48163 VARIANT#
L9            13 ALPHA(W)AMYLASE(5A)BACILLUS(5A)(MUTA? OR VARIANT#)

FILE 'BIOTECHNO'
        189431 ALPHA
          4131 AMYLASE
         19958 BACILLUS
        242571 MUTA?
         41198 VARIANT#
L10           21 ALPHA(W)AMYLASE(5A)BACILLUS(5A)(MUTA? OR VARIANT#)

FILE 'WPIDS'
        194783 ALPHA
          6139 AMYLASE
         14301 BACILLUS
         29612 MUTA?
         28078 VARIANT#
L11           28 ALPHA(W)AMYLASE(5A)BACILLUS(5A)(MUTA? OR VARIANT#)

TOTAL FOR ALL FILES
L12          440 ALPHA(W) AMYLASE(5A) BACILLUS(5A)(MUTA? OR VARIANT#)

=> del his
DELETE ALL L# ITEMS? (Y)/N:y

=> s alpha(w)amylase# and bacillus and (muta? or variant#)
FILE 'MEDLINE'
        558654 ALPHA
         22205 AMYLASE#
          5106 ALPHA(W)AMYLASE#
         52343 BACILLUS
        519956 MUTA?
        114021 VARIANT#
L1           206 ALPHA(W)AMYLASE# AND BACILLUS AND (MUTA? OR VARIANT#)

FILE 'SCISEARCH'
        790556 ALPHA
         18681 AMYLASE#
          8529 ALPHA(W)AMYLASE#
         53718 BACILLUS
        505178 MUTA?
        126730 VARIANT#
L2           320 ALPHA(W)AMYLASE# AND BACILLUS AND (MUTA? OR VARIANT#)
```

```
FILE 'LIFESCI'
        172262 ALPHA
          4722 AMYLASE#
          2856 ALPHA(W)AMYLASE#
         27437 BACILLUS
        229719 MUTA?
         38565 VARIANT#
L3         130 ALPHA(W)AMYLASE# AND BACILLUS AND (MUTA? OR VARIANT#)

FILE 'BIOTECHDS'
         31302 ALPHA
          6040 AMYLASE#
          3810 ALPHA(W)AMYLASE#
         18553 BACILLUS
         46339 MUTA?
         15892 VARIANT#
L4         277 ALPHA(W)AMYLASE# AND BACILLUS AND (MUTA? OR VARIANT#)

FILE 'BIOSIS'
        694816 ALPHA
         29150 AMYLASE#
         10502 ALPHA(W)AMYLASE#
         71451 BACILLUS
        561202 MUTA?
        115575 VARIANT#
L5         254 ALPHA(W)AMYLASE# AND BACILLUS AND (MUTA? OR VARIANT#)

FILE 'EMBASE'
        620236 ALPHA
         16642 AMYLASE#
          3737 ALPHA(W)AMYLASE#
         38236 BACILLUS
        434624 MUTA?
         99436 VARIANT#
L6         140 ALPHA(W)AMYLASE# AND BACILLUS AND (MUTA? OR VARIANT#)

FILE 'HCAPLUS'
       1615721 ALPHA
         47139 AMYLASE#
         19965 ALPHA(W)AMYLASE#
         90542 BACILLUS
        530340 MUTA?
        113151 VARIANT#
L7         510 ALPHA(W)AMYLASE# AND BACILLUS AND (MUTA? OR VARIANT#)

FILE 'NTIS'
         29097 ALPHA
           167 AMYLASE#
            63 ALPHA(W)AMYLASE#
          1732 BACILLUS
         10117 MUTA?
          4679 VARIANT#
L8            1 ALPHA(W)AMYLASE# AND BACILLUS AND (MUTA? OR VARIANT#)

FILE 'ESBIOBASE'
        239105 ALPHA
          4976 AMYLASE#
          2399 ALPHA(W)AMYLASE#
         17897 BACILLUS
        267301 MUTA?
         48163 VARIANT#
L9           87 ALPHA(W)AMYLASE# AND BACILLUS AND (MUTA? OR VARIANT#)

FILE 'BIOTECHNO'
        189431 ALPHA
```

```
          4194 AMYLASE#
          2130 ALPHA(W)AMYLASE#
         19958 BACILLUS
        242571 MUTA?
         41198 VARIANT#
L10        108 ALPHA(W)AMYLASE# AND BACILLUS AND (MUTA? OR VARIANT#)

FILE 'WPIDS'
        194783 ALPHA
          6561 AMYLASE#
          2839 ALPHA(W)AMYLASE#
         14301 BACILLUS
         29612 MUTA?
         28078 VARIANT#
L11        117 ALPHA(W)AMYLASE# AND BACILLUS AND (MUTA? OR VARIANT#)

TOTAL FOR ALL FILES
L12       2150 ALPHA(W) AMYLASE# AND BACILLUS AND (MUTA? OR VARIANT#)

=> s (168 or 169 or 170 or 171 or 172 or 173 or 174)(10a)(residue# or position# or
amino acid# or muta?)
FILE 'MEDLINE'
         15420 168
         12186 169
         23271 170
         12132 171
         12498 172
         11969 173
         13743 174
        199772 RESIDUE#
        225968 POSITION#
        621306 AMINO
       1623422 ACID#
        559178 AMINO ACID#
                 (AMINO(W)ACID#)
        519956 MUTA?
L13       3987 (168 OR 169 OR 170 OR 171 OR 172 OR 173 OR 174)(10A)(RESIDUE#
               OR POSITION# OR AMINO ACID# OR MUTA?)

FILE 'SCISEARCH'
         12570 168
          9403 169
         25791 170
          9426 171
          9967 172
          9852 173
          9865 174
        210642 RESIDUE#
        314076 POSITION#
        391967 AMINO
       1330425 ACID#
        305817 AMINO ACID#
                 (AMINO(W)ACID#)
        505178 MUTA?
L14       3600 (168 OR 169 OR 170 OR 171 OR 172 OR 173 OR 174)(10A)(RESIDUE#
               OR POSITION# OR AMINO ACID# OR MUTA?)

FILE 'LIFESCI'
          2763 168
          1779 169
          4962 170
          1655 171
          1805 172
          1580 173
          1924 174
```

```
          91767 RESIDUE#
          67513 POSITION#
         169268 "AMINO"
         340409 ACID#
         146376 AMINO ACID#
                  ("AMINO"(W)ACID#)
         229719 MUTA?
L15        2256 (168 OR 169 OR 170 OR 171 OR 172 OR 173 OR 174)(10A)(RESIDUE#
                OR POSITION# OR AMINO ACID# OR MUTA?)

FILE 'BIOTECHDS'
           1072 168
            538 169
           1522 170
            569 171
            590 172
            549 173
            629 174
          24669 RESIDUE#
          14859 POSITION#
          67666 AMINO
         151862 ACID#
          62957 AMINO ACID#
                  (AMINO(W)ACID#)
          46339 MUTA?
L16         1301 (168 OR 169 OR 170 OR 171 OR 172 OR 173 OR 174)(10A)(RESIDUE#
                OR POSITION# OR AMINO ACID# OR MUTA?)

FILE 'BIOSIS'
          13158 168
           9271 169
          21867 170
           8724 171
           9557 172
           8869 173
          11272 174
         236840 RESIDUE#
         231817 POSITION#
         525853 AMINO
        1404840 ACID#
         399649 AMINO ACID#
                  (AMINO(W)ACID#)
         561202 MUTA?
L17         4280 (168 OR 169 OR 170 OR 171 OR 172 OR 173 OR 174)(10A)(RESIDUE#
                OR POSITION# OR AMINO ACID# OR MUTA?)

FILE 'EMBASE'
          10554 168
           8482 169
          18261 170
           7722 171
           8168 172
           7625 173
           8442 174
         174970 RESIDUE#
         201464 POSITION#
         429161 "AMINO"
        1439294 ACID#
         324599 AMINO ACID#
                  ("AMINO"(W)ACID#)
         434624 MUTA?
L18         3462 (168 OR 169 OR 170 OR 171 OR 172 OR 173 OR 174)(10A)(RESIDUE#
                OR POSITION# OR AMINO ACID# OR MUTA?)

FILE 'HCAPLUS'
```

```
          40773 168
          27909 169
         126302 170
          26768 171
          32219 172
          28666 173
          31238 174
         649772 RESIDUE#
         529072 POSITION#
        1074575 AMINO
        4658427 ACID#
         682662 AMINO ACID#
                   (AMINO(W)ACID#)
         530340 MUTA?
L19        7424 (168 OR 169 OR 170 OR 171 OR 172 OR 173 OR 174)(10A)(RESIDUE#
                OR POSITION# OR AMINO ACID# OR MUTA?)

FILE 'NTIS'
          1313 168
           942 169
          3234 170
           951 171
          1052 172
          1257 173
          1037 174
         11286 RESIDUE#
         51353 POSITION#
          6985 AMINO
         55378 ACID#
          5127 AMINO ACID#
                   (AMINO(W)ACID#)
         10117 MUTA?
L20          65 (168 OR 169 OR 170 OR 171 OR 172 OR 173 OR 174)(10A)(RESIDUE#
                OR POSITION# OR AMINO ACID# OR MUTA?)

FILE 'ESBIOBASE'
          4654 168
          3372 169
          7795 170
          3203 171
          3511 172
          3261 173
          3631 174
        108636 RESIDUE#
         80920 POSITION#
        181405 AMINO
        404441 ACID#
        158987 AMINO ACID#
                   (AMINO(W)ACID#)
        267301 MUTA?
L21        2310 (168 OR 169 OR 170 OR 171 OR 172 OR 173 OR 174)(10A)(RESIDUE#
                OR POSITION# OR AMINO ACID# OR MUTA?)

FILE 'BIOTECHNO'
          2509 168
          1821 169
          4814 170
          1689 171
          1867 172
          1729 173
          1973 174
         96204 RESIDUE#
         55352 POSITION#
        204625 AMINO
        371908 ACID#
```

```
         173749 AMINO ACID#
                  (AMINO(W)ACID#)
         242571 MUTA?
L22        2629 (168 OR 169 OR 170 OR 171 OR 172 OR 173 OR 174)(10A)(RESIDUE#
                OR POSITION# OR AMINO ACID# OR MUTA?)

FILE 'WPIDS'
           4552 168
           1590 169
          38066 170
           4268 171
           5663 172
           2465 173
           4092 174
         169371 RESIDUE#
        1488938 POSITION#
         249056 AMINO
         992918 ACID#
          89830 AMINO ACID#
                  (AMINO(W)ACID#)
          29612 MUTA?
L23        2217 (168 OR 169 OR 170 OR 171 OR 172 OR 173 OR 174)(10A)(RESIDUE#
                OR POSITION# OR AMINO ACID# OR MUTA?)

TOTAL FOR ALL FILES
L24       33531 (168 OR 169 OR 170 OR 171 OR 172 OR 173 OR 174)(10A)(RESIDUE#
                OR POSITION# OR AMINO ACID# OR MUTA?)

=> s l12 and l24
FILE 'MEDLINE'
L25             4 L1 AND L13

FILE 'SCISEARCH'
L26             4 L2 AND L14

FILE 'LIFESCI'
L27             2 L3 AND L15

FILE 'BIOTECHDS'
L28             7 L4 AND L16

FILE 'BIOSIS'
L29             2 L5 AND L17

FILE 'EMBASE'
L30             2 L6 AND L18

FILE 'HCAPLUS'
L31             9 L7 AND L19

FILE 'NTIS'
L32             0 L8 AND L20

FILE 'ESBIOBASE'
L33             0 L9 AND L21

FILE 'BIOTECHNO'
L34             2 L10 AND L22

FILE 'WPIDS'
L35             3 L11 AND L23

TOTAL FOR ALL FILES
L36            35 L12 AND L24
```

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=> dup rem l36
PROCESSING COMPLETED FOR L36
L37          20 DUP REM L36 (15 DUPLICATES REMOVED)

=> d tot

L37   ANSWER 1 OF 20  BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
TI    Alpha-amylase variants especially for use
      in detergents or cleaning agents have a surface asparagine or glutamine
      residue exchanged to impart improved solvent stability;
         recombinant enzyme purification and solvent stability for use in
         washing liquid and surfactant
AU    BESSLER C; WIELAND S; MAURER K
AN    2006-11372  BIOTECHDS
PI    DE 102004047777 13 Apr 2006

L37  ANSWER 2 OF 20  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI   Alpha-amylase variants especially for use in
     detergents or cleaning agents have a surface asparagine or glutamine
     residue exchanged to impart improved solvent stability.
PI   DE 102004047777 A1 20060413 (200630)*       54    C12N009-26
     WO 2006037484   A2 20060413 (200630)  GE         C12N009-26
        RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IS IT
            KE LS LT LU LV MC MW MZ NA NL OA PL PT RO SD SE SI SK SL SZ TR TZ
            UG ZM ZW
         W: AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE
            DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG
            KM KP KR KZ LC LK LR LS LT LU LV LY MA MD MG MK MN MW MX MZ NA NG
            NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SM SY TJ TM TN TR
            TT TZ UA UG US UZ VC VN YU ZA ZM ZW
IN   BESSLER, C; MAURER, K; WIELAND, S

L37  ANSWER 3 OF 20     MEDLINE on STN                    DUPLICATE 1
TI   Expression of the promoter for the maltogenic amylase gene in
     Bacillus subtilis 168.
SO   Journal of microbiology (Seoul, Korea), (2004 Dec) Vol. 42, No. 4, pp.
     319-27.
     Journal code: 9703165. ISSN: 1225-8873.
AU   Kim Do-Yeon; Cha Choon-Hwan; Oh Wan-Seok; Yoon Young-Jun; Kim Jung-Wan
AN   2005023863     MEDLINE

L37   ANSWER 4 OF 20  BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
TI    Modulating Sec-dependent protein secretion, comprises introducing a
      spoIIIJ or yqjG gene linked to an inducible promoter into a
      Bacillus cell and modulating the expression of the spoIIIJ or
      yqjG gene;
         vector-mediated gene transfer and expression in host cell for strain
         improvement
AU    BRON S; TJALSMA H; VAN DIJL J M
AN    2003-22251  BIOTECHDS
PI    WO 2003060068 24 Jul 2003

L37   ANSWER 5 OF 20  BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
TI    Variant of parent Termamyl-like alpha amylase
      , useful in detergent compositions, for starch liquefaction, ethanol
      production, washing and/or dish washing, and textile desizing;
         recombinant enzyme production, vector expression in host cell,
         polymerase chain reaction and mutagenesis
AU    THISTED T; KJAERULFF S; ANDERSEN C; FUGLSANG C C
AN    2002-12006  BIOTECHDS
PI    WO 2002010355 7 Feb 2002

L37  ANSWER 6 OF 20  HCAPLUS  COPYRIGHT 2006 ACS on STN
TI   Mutant α -amylases with improved
     thermal stability for use in detergents
```

SO Eur. Pat. Appl., 28 pp.
CODEN: EPXXDW
IN Endo, Keiji; Igarashi, Kazuaki; Hayashi, Yasuhiro; Hagihara, Hiroshi; Ozaki, Katsuya
AN 2001:10699 HCAPLUS
DN 134:82718

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI EP 1065277	A1	20010103	EP 2000-111911	20000613
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2001054392	A2	20010227	JP 2000-170517	20000607
CN 1277258	A	20001220	CN 2000-118140	20000609
CN 1560242	A	20050105	CN 2004-10049560	20000609
US 2005181493	A1	20050818	US 2004-988529	20041116

L37 ANSWER 7 OF 20 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
TI New nucleic acid encoding lipid acyl hydrolase proteins having pesticidal activity, derived from rice, soybean, or wheat, for controlling insect infestation and pathogenic infection in transgenic plants.

PI WO 2001036468	A2	20010525 (200137)*	EN 89	C07K014-00
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW				
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW				
AU 2001016129	A	20010530 (200152)		C07K014-00

IN CIGAN, A L

L37 ANSWER 8 OF 20 HCAPLUS COPYRIGHT 2006 ACS on STN
TI Probing structural determinants specifying high thermostability in Bacillus licheniformis α -amylase
SO Journal of Molecular Biology (2000), 301(4), 1041-1057
CODEN: JMOBAK; ISSN: 0022-2836
AU Declerck, Nathalie; Machius, Mischa; Wiegand, Georg; Huber, Robert; Gaillardin, Claude
AN 2000:606606 HCAPLUS
DN 133:173912

L37 ANSWER 9 OF 20 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN
TI Proteome analysis of Bacillus subtilis extracellular proteins: a two-dimensional protein electrophoretic study
SO MICROBIOLOGY-SGM, (JAN 2000) Vol. 146, Part 1, pp. 65-75.
ISSN: 1350-0872.
AU Hirose I; Sano K; Shioda I; Kumano M; Nakamura K; Yamane K (Reprint)
AN 2000:59431 SCISEARCH

L37 ANSWER 10 OF 20 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN DUPLICATE 3
TI Purification and characterization of a truncated Bacillus subtilis alpha-amylase produced by Escherichia coli
SO APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, (FEB 1996) Vol. 44, No. 6, pp. 746-752.
ISSN: 0175-7598.
AU Marco J L (Reprint); Bataus L A; Valencia F F; Ulhoa C J; Astolfi S; Felix C R
AN 1996:138383 SCISEARCH

L37 ANSWER 11 OF 20 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN
TI HYBRID BACILLUS-AMYLOLIQUEFACIENS X BACILLUS -LICHENIFORMIS ALPHA-AMYLASES - CONSTRUCTION, PROPERTIES AND SEQUENCE DETERMINANTS

SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (1 JUN 1995) Vol. 230, No. 2, pp. 481-490.
ISSN: 0014-2956.
AU CONRAD B (Reprint); HOANG V; POLLEY A; HOFEMEISTER J
AN 1995:408127 SCISEARCH

L37 ANSWER 12 OF 20 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
TI Lipase and alpha-amylase variant stabilized against peroxidase system;
Humicola lanuginosa or Bacillus licheniformis enzyme stabilization by enzyme engineering for use in a surfactant composition
AN 1994-11299 BIOTECHDS
PI WO 9414951 7 Jul 1994

L37 ANSWER 13 OF 20 HCAPLUS COPYRIGHT 2006 ACS on STN
TI Cloning and expression of α -amylase from Bacillus subtilis in Escherichia coli and B. subtilis
SO Shengwu Gongcheng Xuebao (1992), 8(4), 334-8
CODEN: SGXUED; ISSN: 1000-3061
AU Xiong, Zhan; Tu, Zhuxin; Zhou, Heshang; Wang, Xiaoping
AN 1993:184581 HCAPLUS
DN 118:184581

L37 ANSWER 14 OF 20 MEDLINE on STN DUPLICATE 4
TI A novel Bacillus subtilis gene involved in negative control of sporulation and degradative-enzyme production.
SO Journal of bacteriology, (1990 Apr) Vol. 172, No. 4, pp. 1783-90.
Journal code: 2985120R. ISSN: 0021-9193.
AU Honjo M; Nakayama A; Fukazawa K; Kawamura K; Ando K; Hori M; Furutani Y
AN 90202692 MEDLINE

L37 ANSWER 15 OF 20 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
TI Starch degrading enzymes of Bacilli: cloning and analyses of the genes and gene products;
Bacillus spp. alpha-amylase, G6-amylase and cyclomaltodextrin-glucanotransferase characterization (conference paper)
SO GIM 90; (1990) Pt.II, 923-34
AU Yamane K; Nakamura A; Kimura K; Takano T; Kobayashi S
AN 1992-03508 BIOTECHDS

L37 ANSWER 16 OF 20 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
TI Nucleotide sequence of the maltohexaose-producing amylase gene from an alkalophilic Bacillus sp. Number 707 and structural similarity to liquefying type alpha-amylase;
gene expression in Escherichia coli and Bacillus subtilis
SO Biochem.Biophys.Res.Commun.; (1988) 151, 1, 25-31
CODEN: BBRCA9
AU Tsukamoto A; Kimura K; Ishii Y; Takano T; *Yamane K
AN 1988-05280 BIOTECHDS

L37 ANSWER 17 OF 20 MEDLINE on STN DUPLICATE 5
TI Isolation and characterization of a cis-acting mutation conferring catabolite repression resistance to alpha-amylase synthesis in Bacillus subtilis.
SO Journal of bacteriology, (1985 Mar) Vol. 161, No. 3, pp. 875-81.
Journal code: 2985120R. ISSN: 0021-9193.
AU Nicholson W L; Chambliss G H
AN 85130820 MEDLINE

L37 ANSWER 18 OF 20 LIFESCI COPYRIGHT 2006 CSA on STN
TI Bacillus subtilis (natto) plasmid responsible for polyglutamate production encoding gamma -glutamyltranspeptidase.
SO J. FAC. AGRIC. KYUSHU UNIV., (1985) vol. 30, no. 2-3, pp. 95-105.

AU Hara, T.; Fujio, Y.; Ueda, S.
AN 85:42822 LIFESCI

L37 ANSWER 19 OF 20 MEDLINE on STN DUPLICATE 6
TI Genes affecting the productivity of alpha-amylase in Bacillus subtilis Marburg.
SO Journal of bacteriology, (1975 Feb) Vol. 121, No. 2, pp. 688-94. Journal code: 2985120R. ISSN: 0021-9193.
AU Sekiguchi J; Takada N; Okada H
AN 75095465 MEDLINE

L37 ANSWER 20 OF 20 HCAPLUS COPYRIGHT 2006 ACS on STN
TI Regulation of α -amylase production in a Bacillus subtilis Marburg strain. I. Isolation of mutants which produce high levels of α -amylase and analysis of their enzymes
SO Hakko Kogaku Zasshi (1972), 50(12), 801-9 CODEN: HKZAA2; ISSN: 0367-5963
AU Sekiguchi, Junichi; Okada, Hirosuke
AN 1973:70185 HCAPLUS
DN 78:70185

=> d ab 1-
YOU HAVE REQUESTED DATA FROM 20 ANSWERS - CONTINUE? Y/(N):y

L37 ANSWER 1 OF 20 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AB DERWENT ABSTRACT:
NOVELTY - alpha-Amylase variants or mutants have an asparagine or glutamine residue on the surface exchanged to Alanine, Cysteine, Phenylalanine, Glycine, Histidine, Isoleucine, Lysine, Leucine, Methionine, Proline, Arginine, Serine, Threonine, Valine, Tryptophan or Tyrosine, are new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for (1) increasing the stability of an alpha-amylase to solvent-induced hydrolysis by having surface residues exchanged as above; (2) nucleic acids coding for the above variants; (3) vectors containing the nucleic acids; and (4) cells containing the above genetically-modified nucleic acids.

BIOTECHNOLOGY - Preferred Variants : Residue Asn is replaced by Ala, Gly, Lys or Arg or especially by Ser or Thr or residue Gln is replaced by Ala, Gly, Ile, Lys, Arg or especially Ser or Thr. Prior to exchanging the amino acid residue has an accessibility of greater than or equal to10 (especially greater than or equal to30)%, the accessibilty being calculated on a scale 0% (not accessible to the solvent to 100% (contained in a hypothetical pentapeptide GGXGG). The number of such amino acids is 2-10, especially 4-6. Preferred Starting Materials: The starting material is an alpha-amylase derived from Bacillus sp A7-7 (DSM 12368) or 707 or (KSM-AP1378) or (KSM-K38) or (MK716) or (TS-23), Bacillus licheniformis, Bacillus stearothermophilus or Bacillus agaradherens, a cyclodextrin-glucanotransferase (CGTase) from Bacillus agaradherens (especially DSM 9948) or a hybrid amylase from an alpha-amylase from Bacillus amyloliquefaciens and from Bacillus licheniformis, especially a hybrid amylase AL34, AL76, AL112, AL256, ALA34-84, LAL19-153 or LAL19-433. In particular, the amino acid sequence of the starting alpha-amylase is greater than or equal to96 (especially 100)% identical to the SEQ ID No 2 amino acid sequence in the positions +1 to 484 or 485 (especially with additional point mutations in one or more of the following positions : 5, 167, 170, 177, 202, 204, 271, 330, 377, 385 and 445 in counting of the mature protein as per SEQ ID No 2) or the starting material is an alpha -amylase variant with increasing 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11 additional point mutations (especially with the

following point mutations : 5A, 167R, 170P, 177L, 202L, 204V, 271D, 330D, 377R, 385S and/or 445Q. Preferred Nucleic Acids: The nucleic acids are derived from a nucleic acid as per SEQ ID NO 1 or from a variant of this with increasing 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11 non-invention additional point mutations. Preferred Vector: The vector is a cloning or expression vector. Preferred Cell: The cell is derived from a bacterium secreting the alpha-amylase in a surrounding medium, especially (i) a gram-positive bacterium of the Bacillus, Staphylococcus or Corynebacterium genus and, in particular, Bacillus lentus, Bacillus licheniformis, Bacillus amyloliquefaciens, Bacillus subtilis, Bacillus globigii, Bacillus alcaliphilis, Staphylococcus carnosus or Corynebacterium glutamicum, with a derivative of Bacillus licheniformis (DSM 13) being especially preferred; or (ii) a gram-negative bacterium of the Escherichia coli, Klebsiella, Pseudomomas or Xanthomonas genus, especially a derivative of Escherichia coli (BL-21(DE3)) or (RV308) or (DH5alpha) or (JM109) or (XL-1) or Klebsiella planticola (Rf). Preferred Process: Stability against hydrolysis caused by high temperatures or high pH values is achieved, especially by use of a vector as above and in particular, by use of a cell as above.

USE - The variant is used in an agent, especially in a compacted, liquid, gel or paste detergent or cleaning agent for textile or hard surface use at 0.000001-5 (especially 0.00001-3) weight% and optionally in encapsulated form, the agent also contains another enzyme such as another amylase, protease, lipase, cutinase, (hemi)cellulase, beta-glucanase, oxidase, peroxidase and/or laccase. The agent is used in dishwashers or washing machines at 0.01-400 (especially 0.03-100) mg and in treatment of raw materials or intermediate products for textile production, especially in desizing cotton. (all claimed).

ADVANTAGE - The variant, especially that derived from Bacillus sp A 7-7 (DSM 12368), has improved stabilty against hydrolysis caused by solvents especially under high temperature or high pH conditions.

EXAMPLE - alpha-Amylase variants of the types surface-modified as described above showed improved solvent stability as compared to their non-modified counterparts in tests involving 30 minutes incubation at 40degreesC in a buffer at pH 11 and also showed improved values in the activity test of WO200210356.(54 pages)

L37 ANSWER 2 OF 20 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN

AB DE1004047777 A UPAB: 20060510

NOVELTY - alpha -Amylase variants or mutants have an asparagine or glutamine residue on the surface exchanged to Alanine, Cysteine, Phenylalanine, Glycine, Histidine, Isoleucine, Lysine, Leucine, Methionine, Proline, Arginine, Serine, Threonine, Valine, Tryptophan or Tyrosine, are new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for

(1) increasing the stability of an alpha -amylase to solvent-induced hydrolysis by having surface residues exchanged as above;

(2) nucleic acids coding for the above variants;

(3) vectors containing the nucleic acids; and

(4) cells containing the above genetically-modified nucleic acids.

USE - The variant is used in an agent, especially in a compacted, liquid, gel or paste detergent or cleaning agent for textile or hard surface use at 0.000001-5 (especially 0.00001-3) weight% and optionally in encapsulated form, the agent also contains another enzyme such as another amylase, protease, lipase, cutinase, (hemi)cellulase, beta -glucanase, oxidase, peroxidase and/or laccase. The agent is used in dishwashers or washing machines at 0.01-400 (especially 0.03-100) mg and in treatment of raw materials or intermediate products for textile production, especially in desizing cotton. (all claimed).

ADVANTAGE - The variant, especially that derived from

Bacillus sp A 7-7 (DSM 12368), has improved stabilty against hydrolysis caused by solvents especially under high temperature or high pH conditions.
Dwg.0/2

L37 ANSWER 3 OF 20 MEDLINE on STN DUPLICATE 1
AB An additional amylase, besides the typical alpha-amylase , was detected for the first time in the cytoplasm of B. subtilis SUH4-2, an isolate from Korean soil. The corresponding gene (bbmA) encoded a maltogenic amylase (MAase) and its sequence was almost identical to the yvdF gene of B. subtilis 168, whose function was unknown. Southern blot analysis using bbmA as the probe indicated that this gene was ubiquitous among various B. subtilis strains. In an effort to understand the physiological function of the bbmA gene in B. subtilis, the expression pattern of the gene was monitored by measuring the beta-galactosidase activity produced from the bbmA promoter fused to the amino terminus of the lacZ structural gene, which was then integrated into the amyE locus on the B. subtilis 168 chromosome. The promoter was induced during the mid-log phase and fully expressed at the early stationary phase in defined media containing beta-cyclodextrin (beta-CD), maltose, or starch. On the other hand, it was kept repressed in the presence of glucose, fructose, sucrose, or glycerol, suggesting that catabolite repression might be involved in the expression of the gene. Production of the beta-CD hydrolyzing activity was impaired by the spo0A mutation in B. subtilis 168, indicating the involvement of an additional regulatory system exerting control on the promoter. Inactivation of yvdF resulted in a significant decrease of the beta-CD hydrolyzing activity, if not all. This result implied the presence of an additional enzyme(s) that is capable of hydrolyzing beta-CD in B. subtilis 168. Based on the results, MAase encoded by bbmA is likely to be involved in maltose and beta-CD utilization when other sugars, which are readily usable as an energy source, are not available during the stationary phase.

L37 ANSWER 4 OF 20 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AB DERWENT ABSTRACT:
NOVELTY - Modulating Sec-dependent protein secretion comprising introducing a spoIIIJ or yqjG gene linked to an inducible promoter into a Bacillus cell, and modulating the expression of the spoIIIJ or yqjG gene by varying the level of induction of the inducible promoter, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for: (1) a purified DNA molecule comprising an inducible promoter operatively linked to the spoIIIJ or yqjG gene; and (2) a method of modulating the secretion of a protein of interest comprising forming a first DNA molecule encoding a chimeric protein comprising a Sec-dependent secretion signal peptide, forming a second DNA molecule encoding an inducible promoter operably linked to the spoIIIJ or yqjG gene, transforming a host cell with the DNA molecule, and growing the host cell under conditions where the protein of interest is expressed at the desired level.

WIDER DISCLOSURE - Methods of inhibiting sporulation in a Bacillus cell comprising a mutation of the spoIIIJ gene, where the mutation results in the formation of an inactive gene product, are also disclosed.

BIOTECHNOLOGY - Preferred Method: Alternatively, modulating Sec-dependent protein secretion comprises providing a Bacillus cell comprising spoIIIJ and yqjG genes linked to an endogenous high expression promoter, and modulating the expression of the spoIIIJ and yqjG genes by varying the level of induction of the promoter. The (inducible) promoter is the Pspac promoter. In modulating the secretion of a protein of interest, the host cell is grown under conditions where the inducible promoter is induced. The protein of interest is expressed at low level.

USE - The methods are useful for enhancing the secretion of proteins from a host cell, preferably from a Bacillus cell, that may be made to be secreted via the Sec-dependent secretion pathway. The DNA

molecules are useful for the inducible expression of the spoIIIJ and/or yqjG genes.

EXAMPLE - To evaluate the importance of yqjG and spoIIIJ function for protein secretion, Bacillus subtilis DELTAyqjG, DELTAspoIIIJ and DELTAyqjG-IspoIIIJ, as well as the parental strain 168 were transformed with plasmid pLip2031 for the secretion of the B. subtilis lipase LipA, pPSPPhoA5 for the secretion of the alkaline phosphatase PhoA of Escherichia coli fused to the prepro-region of the lipase gene from Staphylococcus hyicus, or pKTH10 for the secretion of the alpha-amylase AmyQ. In order to deplete B. subtilis DELTAyqjG-IspoIIIJ of spoIIIJ, this strain was grown for 3 hours in tryptone/yeast extract (TY) medium without isopropyl-beta-D-thiogalacto-pyranoside (IPTG). As a control, TY medium with 50 nM IPTG or 500 nM IPTG was used. The secretion of LipA, PhoA and AmyQ was analyzed by Western blotting. The levels of LipA, PhoA and AmyQ in the medium of spoIIIJ-depleted cells of B. subtilis DELTAyqjG-IspoIIIJ (no IPTG) were significantly reduce compared to those in the media of the fully induced double mutant (500 nM IPTG), or the parental strain 168 . The levels of the LipA and PhoA in the media of DELTAyqjG-IspoIIIJ strains that were fully induced with IPTG (500 nM) were higher than those in the media of the parental control strains. This suggests that over expression of the spoIIIJ gene can result in improved protein secretion in B. subtilis.(50 pages)

L37 ANSWER 5 OF 20 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

AB DERWENT ABSTRACT:

NOVELTY - A variant (I) of a parent Termamyl-like alpha -amylase, comprising an alteration at one or more positions (P), having alpha-amylase activity, is new.

DETAILED DESCRIPTION - (P) includes positions 49, 60, 104, 132, 161, 170, 176, 180, 181, 183, 200, 203, 204, 207, 212, 237, 239, 250, 280, 298, 318, 374, 385, 393, 402, 406, 427, 430, 440, 447 or 482. The alteration(s) are independently selected from an insertion at downstream position of the amino acid at (P), deletion of an amino acid at (P) or substitution of an amino acid at (P) with other amino acid, where each position corresponds to a position of the amino acid sequence of the parent Termamyl-like alpha-amylase comprising a sequence (S1) of 483 amino acids fully defined in the specification. INDEPENDENT CLAIMS are also included for the following: (1) a DNA construct (II) comprising a DNA sequence encoding (I); (2) a recombinant expression vector (III) comprising (II); (3) a cell (IV) which is transformed with (II) or (III); (4) a composition (C) comprising (I); and (5) a detergent composition (DC) comprising (I).

BIOTECHNOLOGY - Preferred Variant: (I) has one or more of the mutations given in the specification using S1 for the numbering. The parent Termamyl-like alpha-amylase is derived from a strain of Bacillus licheniformis (S1), B.amyloliquefaciens (comprising a sequence of 483 amino acids fully defined in the specification) and B.stearothermophilus (comprising a sequence of 515 amino acids fully defined in the specification). The parent Termamyl-like alpha-amylase is selected from SP690, SP722, AA560, 707 alpha-amylase and KSM-AP1378 (all sequences comprising 485 amino acids fully defined in the specification). The parent alpha-amylase has at least 60%, preferably 90% identity to S1, and is encoded by a nucleic acid sequence which hybridizes under low, medium or high stringency conditions with a nucleic acid sequence comprising 1920 nucleotides fully defined in the specification. Preferred Cell: (IV) is a microorganism such as bacterium or fungus. The bacterium is B.subtilis, B.licheniformis, B.lentus, B.brevis, B.stearothermophilus, B.alkalophilus, B.amyloliquefaciens, B.coagulans, B.circulans, B.lautus or B.thuringiensis. Preferred Composition: (C) further comprises B.stearothermophilus (BSG) alpha-amylase in particular SP961, in a ratio of 1:10-10:1, preferably 1:2. (C) further comprises a glycoamylase, pullulanase and/or a phytase. DC further

comprises protease, lipase, peroxidase, amylolytic enzyme glucoamylase, maltogenic amylase, CGTase, mannanase, cutinase, laccase and/or a cellulase. Preparation: The variant is prepared by standard genetic recombinant techniques.

USE - (I) is used for starch liquefaction, ethanol production, washing and/or dish washing, and textile desizing (claimed).

ADVANTAGE - (I) has altered stability in particular at high temperatures from 70-120degreesC and/or low pH in the range from pH 4.0-6.0.

EXAMPLE - To improve the stability at low pH and low calcium concentration of the parent Bacillus licheniformis alpha-amylase, error-prone polymerase chain reaction (PCR) mutagenesis was performed. The plasmid pDN1528 encoding wild-type B.licheniformis alpha-amylase gene was utilized as template to amplify the gene with primers, 22149 (5'-CGATTGCTGACGCTGTTATTTGCG-3') and 2814 (5'-GATCACCCGCGATACCGTC-3') under PCR conditions where increased error rates leads to introduction of random point mutations. The resultant PCR fragment was purified on gel and used in a PCR-based multimerization step with a gel purified vector fragment created by PCR amplification of pDN1528 with primers 24 (5'-GAATGTATGTCGGCCGGCAAAACGCCGGTGA-3') and 27 (5'-GCCGCCGCTGCTGCAGAATGAGGCAGCAAG-3') forming an overlap to the insert fragment. The multimerization reaction was subsequently introduced into B.subtilis. The error-prone library was screened in the low pH filter assay. Clones testing positive upon rescreening was submitted to secondary screening for stability in the liquid assay. (90 pages)

L37 ANSWER 6 OF 20 HCAPLUS COPYRIGHT 2006 ACS on STN

AB The invention relates to mutant α - amylases obtained by making replacement or deletion of at least one of amino acid residues such as the Gln-167, Tyr-169, and Ala-178 in the amino acid sequence of liquefying α -amylase from Bacillus sp. KSM-K38 (FERM BP-6996). A chimeric . alpha.-amylase is also provided comprising the first 21 residues of α -amylase from Bacillus sp. KSM-AP1378 (FERM BP-3048) fused to residues 20-C-terminus of. alpha.-amylase from Bacillus KSM-K38. The mutant α -amylases have excellent properties of high resistance to chelating agents, high specificity in an alkaline medium, and excellent stability to heat, and hence are useful for detergents for automatic dish washer, laundry detergents, and the like.

L37 ANSWER 7 OF 20 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN

AB WO 200136468 A UPAB: 20010704

NOVELTY - An isolated nucleic acid (I), comprising a sequence (S1) of 1479, 1519, 1522, 1463, 1001, 1633 or 980 base pairs, or a sequence insert deposited with American Type Culture Collection (ATCC) Patent Deposit No.PTA 410-415 or PTA-644, encoding a lipid acyl hydrolase (LAH) polypeptide with a sequence (S2) of 406, 405, 407, 410, 290, 224, 173 or 275 amino acids, given in the specification, is new.

DETAILED DESCRIPTION - A new isolated nucleic acid molecule (I), comprises a nucleotide sequence (S1) of 1479, 1519, 1522, 1463, 1001, 1633 or 980 base pairs, or a sequence insert deposited with American Type Culture Collection (ATCC) in a bacterial host assigned Patent Deposit No.PTA 410-415 or PTA-644, encoding a lipid acyl hydrolase (LAH) polypeptide having a sequence (S2) of 406, 405, 407, 410, 290, 224, 173 or 275 amino acids, given in the specification. Alternatively, (I) comprises a nucleotide sequence that shares 70 % sequence identity with (S1), a nucleotide sequence comprising 16 contiguous nucleotides of (S1), a sequence hybridizable with (S1) or a nucleotide sequence encoding LAH having pesticidal activity which differs from (I) due to degeneracy of the genetic code.

INDEPENDENT CLAIMS are also included for the following:

(1) a DNA construct (II) comprising (I) operably linked to a promoter that is functional in a plant cell;
(2) a vector comprising (II);
(3) a transformed plant or plant cell comprising (II);
(4) a purified LAH polypeptide (III); and
(5) a purified polypeptide having pesticidal activity, consisting of a sequence having 70 %, 80 %, 90 % or 95 % identity to (S2).
ACTIVITY - Insecticidal. Test details are described but no results given.
MECHANISM OF ACTION - Lipid acyl hydrolase.
USE - (I) is useful in the genetic manipulation of plants and for transforming bacteria, fungi, yeast and other organisms. (I) and a polypeptide (III) encoded by (I) are useful for controlling insect infestation and pathogenic infections in transgenic plants. (I) confers pesticidal resistance to organisms into which it has been introduced and is also useful in mutagenic and recombinogenic protocols, to produce polypeptides with improved biological activities. The DNA and protein sequences having pesticidal activity are useful for protecting plants, in particular agricultural crops from pests, including insects, fungi, bacteria, nematodes, viruses or viroids and in particular insect pests. (I) is useful in DNA shuffling protocols for generating libraries of polynucleotides having a desired characteristic. (III) can be used alone or in combination with other proteins or agents from Bacillus, including delta -endotoxin and vegetative insecticidal proteins, protease inhibitors, lectins, alpha -amylases , peroxidases and cholesterol oxidase, to control different insect pests. Dwg.0/9

L37 ANSWER 8 OF 20 HCAPLUS COPYRIGHT 2006 ACS on STN
AB B. licheniformis α -amylase (BLA) is a starch-degrading enzyme that is highly thermostable although it is produced by a rather mesophilic organism. Over the last decade, the origin of the BLA thermal properties was extensively investigated in both academic and industrial labs., yet it is still poorly understood. Here, the authors used structure-based mutagenesis in order to probe the role of amino acid residues previously proposed as being important for BLA thermostability. Residues involved in salt-bridges, Ca2+ binding, or potential deamidation processes were selected and replaced with various amino acids using a site-directed mutagenesis method, based on informational suppression. A total of 175 BLA variants were created and analyzed in vitro. Active BLA variants were tested for thermostability by measuring residual activities after incubation at high temperature Out of the 15 target residues, 7 (Asp-121, Asn-126, Asp-164, Asn-192, Asp-200, Asp-204, and Ala-269) were found to be particularly intolerant to any amino acid substitutions, some of which led to very unstable mutant enzymes. By contrast, three Asn residues (Asn-172, Asn-188, and Asn-190) could be replaced with amino acid residues that significantly increased the thermostability compared to the wild-type enzyme. The highest stabilization event resulted from the substitution of Phe in place of Asn at position 190, leading to a 6-fold increase of the enzyme half-life at 80° (pH 5.6, 0.1 mM CaCl2). These results, combined with those of previous mutational analyses, showed that the structural determinants contributing to the overall thermostability of BLA were concentrated in domain B and at its interface with the central A domain. This region contained a triadic Ca-Na-Ca metal-binding site that appeared extremely sensitive to any modification that might alter or reinforce the network of electrostatic interactions entrapping the metal cations. In particular, a loop spanning from residue 178 to 199, which undergoes pronounced conformational changes upon removal of Ca2+, appeared to be the key feature for maintaining the enzyme's structural integrity. Outside of this region, most salt-bridges that were destroyed by mutations were found to be dispensable, except for an Asp-121-Arg-127 salt-bridge that contributed to the enhanced thermostability of BLA compared to other homologous bacterial α -amylases. Finally, these

studies demonstrated that the natural resistance of BLA against high temperature
was not optimized and could be enhanced further through various means, including the removal of possibly deamidating residues. (c) 2000 Academic Press.

L37 ANSWER 9 OF 20 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

AB To analyse the proteome of Bacillus subtilis extracellular proteins, extracellular protein samples were prepared from culture media (minimal medium containing 0.4% glucose) of parental a. subtilis 168, a secA-temperature sensitive mutant and an ffh conditional mutant, and examined by two-dimensional gel electrophoresis. Approximately 100 to 110 spots were visualized in a gel of B. subtilis 168 extracellular proteins. Over 90% and 80% of these disappeared in the absence of SecA and Ffh, respectively. Thirty-eight obvious spots on the gel of the B. subtilis 168 preparation were selected and compared with spots obtained under SecA- or Ffh-deficient conditions. The appearance of 36 of these 38 spots depended on SecA and Ffh. Nineteen additional extracellular proteins were detected in cultures maintained in cellobiose, maltose and soluble starch. Among 23 proteins of which the N-terminal amino acid sequences were determined, 17 were extracellular proteins having signal peptides in their precursor form. Two membrane proteins, Yfnl and YflE, were cleaved behind (226)Ala-Tyr-Ala(228) and (213)Ala-Leu-Ala(215). respectively, and of which products seemed to be liberated into the culture medium. The production of Yfnl and YflE were also dependent on SecA and Ffh. These results indicate that most extracellular proteins target to and translocate across the cytoplasmic membrane by co-operation between the signal-recognition particle and Sec protein-secretion pathways. In contrast, a spot for Hag appeared independent from SecA and Ffh. Intracellular proteins Gap, SodA and KatA were identified in the extracellular protein samples. On the basis of these results and computer searches, it was predicted that B. subtilis produces 150 to 180 proteins extracellularly.

L37 ANSWER 10 OF 20 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN DUPLICATE 3

AB A Bacillus subtilis amylase gene was inserted into a plasmid which was transferred to Escherichia coli. During cloning, a 3' region encoding 171 carboxyterminal amino acids was
replaced by a nucleotide sequence that encoded 33 amino acid residues not present in the indigenous protein. The transformed cells produced substantial amylolytic activity. The active protein was purified to apparent homogeneity. Its molecular mass (48 kDa), as estimated in sodium dodecyl sulfate/polyacrylamide gel electrophoresis, was lower than the molecular mass values calculated from the derived amino acid sequences of the B. subtilis complete alpha-amylase (57.7 kDa) and
the truncated protein (54.1 kDa). This truncated enzyme form hydrolysed starch with a K-m of 3.845 mg/ml. Activity was optimal at pH 6.5 and 50 degrees C, and the purifed enzyme was stable at temperatures up to 50 degrees C. While Hg2+, Fe3+ and Al+3 were effective in inhibiting the truncated enzyme, Mn2+ and Co2+ considerably enhanced the activity.

L37 ANSWER 11 OF 20 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

AB A series of 33 single and mosaic hybrid alpha-amylases was constructed from the genes amyBA or amyLI, encoding the alpha-amylases from Bacillus amyloliquefaciens (AmyBA) and Bacillus licheniformis (AmyLI). The hybrid proteins, consisting of the entire alpha-amylase sequence with a variable portion of AmyBA or AmyLI origin, were characterized in order to find enzymes with new properties (thermostability, temper ature and pH optima, and substrate specificity), and to localize the amino acid sequence regions responsible for the changes.

The thermostability of the AmyBA/AmyLI (AL-type) hybrid proteins correlated with the position and the length of the hybrid sequence. The hybrid enzymes fell into six groups retaining, in comparison to AmyBA, a certain value of the extra-thermostability of AmyLI or becoming more thermolabile than AmyBA. Four regions are proposed to contain thermostability determinants (TSDs). They map between amino acid residues 34-76, 112-142, 174-179 and 263-276 of the respective hybrid enzymes, indicating the dominance of the N-terminal half of AmyLI for these hybrid enzymes' resistance against irreversible inactivation. Two (TSD3 and TSD4) coincide with regions I and II that had already been suggested to stabilise AmyLI [Suzuki, Y., Ito, N., Yuuki, T., Yamagata, H. and Udake, S. (1989) J. Biol. Chemical 264, 18 933 -18 938]. The temperature dependence of activity of the AL-type hybrid alpha-amylases was compared at pH 6.4 and pH 7.6 and the hybrid enzymes of one thermostability group were found to have similar temperature responses.

A hybrid region between residues 34-76 is demonstrated to correlate with the alpha-amylases' substrate specificity, i.e. either hydrolysis or accumulation of maltohexaose. This region was therefore named the G6G5 region. The exchange of internal sequences between residues 17-201 of AmyBA by the AmyLI counterpart in ALA-type mosaic hybrid alpha-amylases, with one exception (ALA99-429), unexpectedly destabilized the respective ALA-type hybrids. Two of these hybrid alpha-amylases (ALA17-151 and ALA76-151) were less thermostable than AmyBA, while others (ALA112-151, ALA112-201) were enzymically inactive. These data support specific roles of the predicted A1-B domain portion between residues 17-201 of those Bacillus alpha-amylases probably for correct folding and enzymic activity.

L37 ANSWER 12 OF 20 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

AB A new lipase (EC-3.1.1.3) or alpha-amylase (EC-3.2.1.1) variant, stabilized towards inactivation caused by a peroxidase (EC-1.11.1.7) system (a peroxidase, an H2O2 source and an enhancing agent) has at least 1 native Tyr residue deleted or substituted with Phe, Leu, Ile, Val, Gln, Asn, Ser, Thr, Glu or His. The lipase variant is from Humicola lanuginosa (preferred), Humicola brevispora, Humicola brevis var. thermoidea, Humicola insolens, Pseudomonas cepacia, Pseudomonas fragi, Pseudomonas stutzeri, Pseudomonas fluorescens, Fusarium oxysporum, Rhizomucor miehei, Candida antarctica or Candida cylindracea, and has a mutation at position 16, 21, 53, 138, 164, 171, 194, 213, 220 or 261. The alpha-amylase variant is from Bacillus licheniformis (preferred) or Aspergillus sp., and has a mutation at position 10, 14, 31, 46, 56, 59, 62, 77, 98, 150, 158, 175, 193, 195, 198, 203, 219, 262, 273, 290, 302, 348, 358, 363, 367, 394, 396, 402, 439 or 480. The enzymes are useful in surfactant compositions, and have improved compatibility with peroxidase systems used to inhibit dye transfer. (28pp)

L37 ANSWER 13 OF 20 HCAPLUS COPYRIGHT 2006 ACS on STN

AB With a mutant Bacillus subtilis 168 as donor and plasmid pBE1 as vector, the α -amylase gene was cloned and expressed in E. coli. This α -amylase gene was situated in a 7367 bp BglII restriction fragment, which was then subcloned and expressed in Bacillus subtilis by plasmid pUB110 as vector. Glucose repressed the synthesis of . alpha.-amylase.

L37 ANSWER 14 OF 20 MEDLINE on STN DUPLICATE 4

AB We have cloned a 2.5-kilobase fragment of the Bacillus subtilis genomic DNA which caused the reduction of extracellular and cell-associated protease levels when present in high copy number. This fragment, in multicopy, was also responsible for reduced levels of alpha-amylase, levansucrase, alkaline phosphatase, and

sporulation inhibition. The gene relevant to this pleiotropic phenotype is referred to as pai. By DNA sequencing, two open reading frames--ORF1 and ORF2, encoding polypeptides of 172 and 207 amino acid residues, respectively--were found. These open reading frames seemed to form an operon. Deletion analysis revealed that an entire region for ORF1 and ORF2 was necessary for the pai phenotype. In addition, it was observed that the presence of the pai gene, in multicopy, caused overproduction of two proteins (molecular masses, 21 and 24 kilodaltons [kDa]). Analyses of the N-terminal amino acid sequences of these two proteins suggested that they were products of ORF1 and ORF2. Disruption of the pai gene at ORF1 in the genomic DNA resulted in the release of repression on protease synthesis and sporulation in glucose-enriched (2%) medium. The mutant carrying insertional disruption at ORF2 could not be constructed, suggesting that the ORF2 product, the 24-kDa protein, is essential for growth. The 21-kDa protein contains a helix-turn-helix domain observed in other DNA-binding proteins. Chromosomal mapping of pai indicated that this gene is located close to thr-5. These results suggest that the pai gene is a novel transcriptional-regulation gene involved in glucose repression.

L37 ANSWER 15 OF 20 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

AB Structural relationships between alpha-amylase (EC-3.2.1.1), malto-oligosaccharide-producing amylase (Gn-amylase) and cyclomaltodextrin-glucanotransferase (CGTase, EC-2.4.1.19) in Bacillus spp. were characterized. Most alpha-amylases contain about 500 amino acids, with 3 conserved regions (A, B and C), about 100, 200 and 300 residues from the N-terminus, respectively, which are considered to form the active center, with a critical Glu residue (e.g. Glu-208 in the Bacillus natto enzyme). G6-amylase from alkalophilic Bacillus sp. 707 shows homology to liquefying amylases. The extracellular alpha-amylase of Bacillus subtilis 168 contains 619 amino acids, and has about 200 non-essential C-terminal amino acids. CGTases degrade starch and amylose to cyclodextrins. 3 Conserved regions and a Glu residue similar to those in alpha-amylase are present in CGTases, but an additional C-terminal cyclization domain of about 350 amino acids is present. Cloning and deletion or site-directed mutagenesis of CGTase genes (e.g. from Bacillus sp. 1011) has resulted in a model for cleavage and reconstitution of alpha-1,4-bonds. (18 ref)

L37 ANSWER 16 OF 20 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

AB The nucleotide sequence of the gene for maltohexaose- producing amylase (G6-amylase) from an alkalophilic Bacillus sp. Number 707 was determined. A 4.4 kb DNA fragment encoding the G6- amylase gene was inserted into plasmid pBR322 to give plasmid pTUE306 which was maintained in E. coli HB101. The Bacillus subtilis plasmid pTUB812 (7.3 kb) and the G6-amylase gene was expressed in an alpha-amylase (EC-3.2.1.1)-deficient mutant of B. subtilis 168, B. subtilis 207-25. The crude amylase preparation obtained from the culture medium of B. subtilis 207-25 was the sole amylase component and had a mol.weight of 58,000. The same enzyme was also detected in the extracellular and periplasmic fractions of E. coli harboring pTUB306. The DNA sequence of the encoding gene started at an ATG initiation codon, and contained an open reading frame of 1,554 bp. The N-terminal portion encoded a 33 amino acid-long signal peptide. The amino acid sequence of the extracellular mature enzyme was over 60% homologous to those of the liquefying type alpha-amylases. (14 ref)

L37 ANSWER 17 OF 20 MEDLINE on STN DUPLICATE 5

AB Bacillus subtilis 168GR10 was shown to contain a mutation, gra-10, which allowed normal temporal activation of alpha-amylase synthesis in the presence of a concentration of glucose that is inhibitory to activation of amylase

synthesis in the parent strain, 168. The gra-10 mutation was mapped by phage PBS-1-mediated transduction and by transformation to a site between lin-2 and aroI906, very tightly linked to amyE, the alpha-amylase structural gene. The gra-10 mutation did not pleiotropically affect catabolite repression of sporulation or of the synthesis of extracellular proteases or RNase and was unable to confer glucose-resistance to the synthesis of chloramphenicol acetyltransferase encoded by the cat-86 gene driven by the amyE promoter region (amyR1) inserted into the promoter-probe plasmid pPL603B. It therefore appears that gra-10 defines a cis-regulatory site for catabolite repression, but not for temporal activation, of amyE expression. The evidence shows that temporal activation and glucose-mediated repression of alpha-amylase synthesis in B. subtilis 168 are distinct phenomena that can be separated by mutation.

L37 ANSWER 18 OF 20 LIFESCI COPYRIGHT 2006 CSA on STN

AB Gamma-glutamyltranspeptidase (gamma -GTP) activity of PGA-producing strains derived by DNA-mediated transformation was significantly increased compared with that of protease, alpha -amylase, and alkaline phosphatase in the PGA-nonproducing recipient strain, B. subtilis Marburg 168. Its enzyme activity was deficient in the stringy-negative mutants cured with acridine orange. Parental strains were found to possess a plasmid, but PGA-nonproducing derivatives were missing the plasmid. The molecular weight of the plasmid was estimated to be 5.7 kilobase (kb) and restriction endonuclease cleavage site map was constructed. It was concluded that the function of the 5.7-kb plasmid detected in B. subtilis (natto) is concerned with PGA production. This observation suggests that a broader range of functions are associated with palsmid in Bacillus species.

L37 ANSWER 19 OF 20 MEDLINE on STN DUPLICATE 6

AB Genetic control of alpha-amylase (alpha-1,4-glucan glucanohydrolase, EC 3.2.1.1.) production by Bacillus subtilis 168 was studied from the standpoint that alpha-amylase production by bacteria is dependent on a long-lived messenger ribonucleic acid and obeys the following equation: E = kappa integral of X-DT where x = cell mass at time t, E = alpha amylase produced, t = culture time, and kappa = productivity constant. So a productivity constand (kappa) is obtained from the slope of the straight line plot of alpha-amylase formed versus the total mass of cells accumulated over that time during the culture process. The following results were obtained. (i) Two sequential mutants, derived from the 168(kappa = 20) strain and having improved alpha-amylase productivity (168 leads to 196), were analyzed for their serine and metal protease production. Strain 128 (kappa = 40) produced half the amount of both proteases, but strain 196 (kappa = 60 similar to 80) produced 20 times that in the original strain. (ii) Amy+ transformants, using the 196 strain as the other three had higher productivity (kappa = 37 similar to 46). These transformants (J71, J47, groups. Seventy-one of 74 Amy+ transformants had a kappa value of 21.0 plus or minus 2.1 and the other three had higher productivity (kappa = 37 similar to 46). These transformants (J71,J47, and J10) produced levels of serine and metal proteases 20 times higher than the other transformants. (iii) Strains 196, J71, J47, and J10 were found to be nonmotile and resistant to phage PBS1, whereas other strains, including strains 168, 128, 3 revertants of strain J71 and 2 revertants of strain 196, were all motile and sensitive to the phage. (iv) Strains 196 and J71 were nonflagellated under electron microscopic observation but strain 168, 128 and a revertant of J71 were flagellated. From the above experimental results, the existence of a quality controlling gene (amyB) was deduced, which is loosely linked to the structural gene and controls productivities of alpha-amylase and proteases, and flagellation. The probable existence of another regulatory gene, amyC, is also discussed.

L37 ANSWER 20 OF 20 HCAPLUS COPYRIGHT 2006 ACS on STN

AB An improvement in the α -amylase productivity of B. subtilis 168 try- (a Marburg strain) was obtained by uv irradiation and selection. Its mechanism was analyzed from the standpoint that . alpha.-amylase production by bacteria is depended on a long lived messenger RNA. The productivity can be evaluated quant. independently from the cell growth. The original Marburg strain 168 and a mutant, 196, having .apprx.3-fold greater productivity, had the same life time for messenger RNA, but their efficiency constants differed by a factor of 3. The difference in efficiency consts. is not caused by an alteration of the enzyme protein, judging from some characteristics of the purified enzyme produced by the original and the improved mutant strains. They are the specific activity, electrophoretic pattern, heat stability, pH-activity profile, and the activity ratio toward soluble starch and p-nitrophenyl-α-D-maltoside (PNPM). Other mechanisms possibly affecting the efficiency value were discussed. α -Amylase produced by the Marburg strain was purified to an acrylamide gel electrophoretically homogeneous state and the α -amylases produced by the original and the mutant strain 196 were found to be identical.

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=> log y

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